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TOWNSEND and TOWNSEND and CREW LLP

By:

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10-28-99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

JOFUKU, et al.

Serial No.: 08/879,827

Filed: June 20, 1997

For: METHODS FOR IMPROVING SEEDS

Examiner: M. Mosher.

Art Unit 1643

Declaration of
Nickolai N. Alexandrov
Under 37 CFR § 1.132

San Francisco, California 94111-3834
September 20, 1999

Commissioner of Patents and Trademarks
Washington, D.C. 20231

I, Nickolai N. Alexandrov, do hereby state and declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

I have studied genetics and molecular biology for 15 years. I received my Ph.D. in 1989 from The Institute for Genetics of Microorganisms, Moscow, Russia, in Molecular Biology. I am currently

Manager of Bioinformatics at Ceres, Inc. in Malibu, California. Ceres is a licensee of the-above referenced application. A copy of my curriculum vitae is attached as Exhibit A.

I have reviewed and understand the contents of the pending claims of Application No. 08/879,827. I have also reviewed and understand the contents of Application No. 08/879,827 as well as the Office Action mailed on April 14, 1999, which rejected claims of Application No. 08/879,827 referring to polypeptides related by percent identity. As explained below, as of the filing date of June 20, 1997, one of ordinary skill in the art would have been enabled to use a sequence alignment computer algorithm, such as BLAST, to determine the percent sequence identity between two polypeptide or nucleotide sequences.

As of June 20, 1997, version 1.4 of the BLAST sequence alignment computer algorithm was available via email (*blast@ncbi.nlm.nih.gov*). Thus, any email user could access the BLAST sequence alignment computer algorithm at the National Center for Biotechnology Information (NCBI). The BLAST algorithm is based on the statistical methods described by Altschul, J. Mol Biol. 219:555-65 (1991) and Altschul, J. Mol. Evol. 36:290-300 (1993). Therefore, the results of a sequence comparison using the BLAST algorithm available at the email address above (*blast@ncbi.nlm.nih.gov*) as of June 20, 1997 would be identical to results from any other available source of the BLAST algorithm set to the same parameter specifications. The operation and default parameters of BLAST v1.4 were available by email (type "help" in the body of the message and mail to: *blast@ncbi.nlm.nih.gov*) as of June 20, 1997. Currently the BLAST v1.4 manual is available on the internet (*e.g.*, http://blast.wustl.edu/blast-1.4/blast1.pdf) or may be accessed by email by typing "help" in the body of the message and e-mailing to: ftp://ncbi.nlm.nih.gov/blast/documents /blemail.txt). The manual as available on the internet is attached as Exhibit B. The default (i.e. standard) parameters disclosed in the manual include the default scoring matrix for the blastp function (BLOSUM62), the expect default (10), and the cutoff or "S" parameter (calculated from the expect parameter). BLAST v1.4

did not use a filter to reduce non-specific, low-complexity sequences. BLAST v1.4 does not allow for gaps within sequences.

Exhibit C provides a typical comparison using BLAST v1.4 using the above-described standard parameters to determine percent identity between SEQ ID NO:4, i.e. one of the AP2 domains disclosed in the specification of Application No. 08/879,82, and the "nr" database. The "Ungapped BLAST search" (i.e. BLAST v1.4) was chosen from a NCBI web page (http://www.ncbi.nlm.nih.gov /BLAST/). From the "Advanced BLAST" page (http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-blast?Jform=1), the "blastp" program was selected to compare a polypeptide sequence to an polypeptide database. Default parameters were set for all parameters except "filter," which was set to "none." The polypeptide query sequence of SEQ ID NO:4 was entered into the data entry box and the query was submitted. The resulting blastp results display a number of polypeptides that are related to SEQ ID. NO:4 and displays their sequence identity. For instance, in the first sequence displayed, one subsequence of the "FLORAL HOMEOTIC PROTEIN APETALA2" was 100% identical with SEQ ID NO:4. In general, at least one alignment is displayed for each related protein, including percentage identity relative to the queried polypeptide sequence.

Because BLAST v1.4 does not use a gapping function when comparing sequences, BLAST v1.4 may break a particular sequence into separate parts and display an alignment and separate percent identity for each part of the sequence. If this occurs, percent identity to the entire query sequence can be calculated by weight averaging the percent identity for each part. For example, if the entire query sequence is divided into two parts by the BLAST algorithm such that three quarters of the entire query sequence is 60% identical to the target sequence and the other one quarter of the query is 20% identical to the target sequence, then the weighted average of the sequence identity would be 50% (0.75x60% +0.25 x 20%=50%).

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Exhibit C demonstrates the basic steps of determining sequence identity between two protein sequences using BLAST v1.4. These steps for sequence comparison were known to those of ordinary skill in the art at the filing date of Application No. 08/879,827. Therefore the disclosure of a query sequence, *e.g.*, an AP2 domain, and a stated percent identity would have enabled one of ordinary skill in the art to determine whether other polypeptide sequences were at least as identical to the query sequence as the stated percent identity.

Date: 10-13-99 N. Alexandrov

Nickolai N. Alexandrov